BULETIN Penyelidikan PPSP SMK
Vol. 4-No.2/3
August/Dicember 2004
For Internal Circulation Only
RESEARCH BULLETIN OF SCHOOL OF MEDICAL SCIENCES, UNIVERSITI SAINS MALAYSIA, HEALTH CAMPUS

INVITED EDITORIAL

A BREAKTHROUGH IN NPC DIAGNOSIS – FROM CONVENTIONAL BIOPSY TO EBV AMP KIT – *(The kit won the gold medal best invention for I-Tex 2004)*

DR. SHAHID HASSAN,
Department of ORL, School of Medical Sciences, Universiti Sains Malaysia Health Campus

Nasopharyngeal carcinoma (NPC) is the second highest cancer amongst men in Malaysia (Lung Cancer being the first). Epstein Barr virus (EBV) is associated with all the types of NPC. In Peninsular Malaysia, the incidence of NPC is 365 cases per year or 5.4% of malignant tumors of nasopharynx (Prasad and Rampal, 1992); followed by lymphomas, adenocarcinomas and adenoid cystic carcinomas. It is potentially a curable disease if detected early in stage I or II; however in the advance stage, five years survival rate drastically falls from 100-80% (in stage I and II) to 58-38% (in stage III and IV). The diagnosis is sometimes delayed with increasing morbidity and mortality for non-representative biopsy, submucosal disease and occult primaries.

The conventional diagnostic method by nasopharyngeal biopsies and fine needle aspiration (FNA) of neck lumps have high rate of false negatives. EBV DNA in tumor cells detected by polymerase chain reaction (PCR) has been shown to be more reliable and able to detect the cancer early before the clinical manifestation. The author and his team in USM had successfully conducted a study under IRPA short-term grant to evaluate the validity and reliability of detecting EBV genes in FNA and biopsy tissue of NPC by PCR. Adequate sample of EBV products $EBNA_1$ and $EBNA_2$ and LMP_1 that are generally implicated in oncogenesis, together with Beta-actin were analyzed in this study conducted for the first time in Southeast Asia. This was a forward step towards the ultimate diagnosis of prospective NPC, otherwise it may lead to an advance disease with regional metastasis and adverse prognosis if diagnosed late. NPC is a unique cancer mostly presented with huge primary and regional metastasis, thus it demands an early diagnosis for

Continued on page 3

IN THIS ISSUE

A Breakthrough in NPC Diagnosis ... 1, 3
From The Editor .. 2
Short Communication ... 4
Departmental Research Activities .. 5
R & D Activities in PPSP . 6 - 10
Abstracts of The Selected Papers . . 11 - 13
Research News Update .. 13 - 15
Research Personality: Assoc Prof (Dr) Mustaffa Musa .. 16, 15

with regional metastasis and adverse prognosis if diagnosed late. NPC is a unique cancer mostly presented with huge primary and regional metastasis, thus it demands an early diagnosis for better prognosis.

This and a few other similar studies have given new hope towards the accurate diagnosis of NPC which may have pitfalls in detection by conventional methods based on histopathology.

Application of this Study Test in Clinical Practice

This study investigated the presence of EBV DNA in metastatic neck of unknown primaries, i.e. NPC. The outcome of this study suggests that EBV DNA detection is a highly sensitive and specific technique in diagnosing metastatic NPC and therefore may be used to guide the diagnostic workup of occult primaries. This will necessitate a careful repeat biopsy technique under direct vision from an anatomically occult area of nasopharynx. Further studies may be required to detect the rate of EBV DNA detection in other tumors like Hodgkin's, Burkih's and B-cell lymphoma and lympho-epithelial carcinoma of salivary gland from the Head and Neck region which are rarely seen in Malaysia. However the scarcity of such cases makes it difficult to obtain tissue and investigate the conditions associated with EBV.

Histopathology remains the gold standard for diagnosis of NPC in the primary site, though it is not without limitations as discussed above. EBV DNA detection is especially useful when HPE fails to detect the malignancy. In clinically doubtful cases, the detection of EBV DNA should raise the index of suspicions leading to a prompt repeat of biopsy under GA if necessary. In non-suspicious cases EBV DNA may indicate pre-invasive stage to be followed up closely with repeat biopsy every 3 months. In Malaysia, the presence of EBV DNA in occult primaries will strongly suggest NPC if lymphoma has been ruled out by cytology, as NPC is very common here; especially in Chinese and Malays it is almost certain that occult primary with EBV DNA has originated from the nasopharynx.

Future Application – Developing a Diagnostic Kit

After obtaining the results from this statistically significant study with a large sample size which has proven the presence of EBV DNA in NPC, we can proceed to optimize this test. This will help us creating a diagnostic kit that is cheap and simple to use as compared to a PCR based test which requires cold storage, reagent transportation and repeated freezing and thawing that lead to erroneous results.

To create EBV amplification (EBV Amp) diagnostic kit, the test needs to be converted to multiplex form, i.e. all genes are simultaneously amplified in a single tube. This is feasible as all 4 genes have discretely different product size and can easily be discerned on ethidium bromide-stained electrophoresis gel. We have finally developed the EBV Amp Kit that contains thermo-stabilized PCR reagents in dry powder form in 0.5ml tube, which can be easily transported without cold chain. The test has been proven to be 97% sensitive and 100% specific for EBV. The amplified product sizes are EBNA1, EBNA2, LMP1 and Beta human actin gene, and an internal control. DNA extraction from tissue performed in standard way can be added to thermo-stabilized PCR mix tube and operated in PCR machine to be finally analyzed on UV trans-illuminator. The result obtained is interpreted in a specified manner. It is hoped that this single tube DNA test for rapid Epstein-Barr virus detection will prove a breakthrough in early diagnosis of NPC which is mandatory for a possible cure. After being patented (in process) EBV Amp kit will be available for general use in NPC practice at reasonable price together with detail of procedure, analysis of PCR product, interpretation criteria and precaution to be taken while performing the test.

Acknowledgements

1. To the team involved in this project including Dr.Yap Yoke Yeow, Dr.M.Ravichandran, Dr. P.Lalitha, Ms.Chan Yean-Yean and Ms.Melissa Chan.
2. To Universiti Sains Malaysia for funding this project through short term IRPA grant.

EBV DNA DETECTION IN THE DIAGNOSIS OF NASOPHARYNGEAL CARCINOMA

Yoke-Yeow Yap[†], Shahid Hassan[‡], M Ravichandran[‡], Melissa Li-Ann Chan[‡], Pua Kin Choo[°]

ABSTRACT

Introduction
Nasopharyngeal carcinoma (NPC) is common in Malaysia but diagnosis is sometimes delayed. The Epstein-Barr virus (EBV) is known to be associated with NPC.
Objective
This study evaluates the validity and reliability of detecting three genes – EBNA1, EBNA2 and LMP1 in nasopharyngeal biopsies and fine-needle aspirates of metastatic neck nodes in NPC.
Materials and methods
Tissue from 72 nasopharyngeal biopsies were collected.Thirty-six were positive and 36 negatives served as controls. Tissue from 70 fine-needle aspirations were similarly obtained. Thirty-five belonged to NPC-positive patients, and 35 of other pathologies served as controls. DNA was extracted, amplified with forward and reverse primers for EBNA1, EBNA2, and LMP1 genes, and human β-actin gene to ensure sufficient DNA for analysis, and detected by electrophoresis. Cloned DNA from B95-8 cell lines served as positives control. Histopathological-proven primary tumour and clinico-pathological criteria for neck nodes were used as gold standard for comparison
Results
35/36 positive nasopharyngeal biopsies contained sufficient DNA. $P > 0.05$ by showed no significant difference from histopathology. EBNA1 has the best sensitivity (97.1%) and specificity (100%) (kappa = 0 97). One patient with obvious nasopharyngeal tumour was negative on the 1st biopsy and confirmed on repeat biopsy 2 weeks later, but EBV DNA was detected in both specimens.
35/36 metastatic NPC specimens contained sufficient DNA. $P > 0.05$ showed no significant difference from clinico-pathological criteria for neck metastasis. A cut-off point of >0/3 genes offrers the highest sensitivity (97.1%) and specificity (94.1%) (kappa = 0.91). All histological types of NPC contained EBV DNA.
Conclusion
EBV DNA detection is reliable and accurate in diagnosing NPC. It is on par with histopathology and superior to fine-needle cytology and can suggest NPC in occult primaries

---

[†] Universiti Putra Malaysia
[‡] Universiti Sains Malaysia
[‡] Universiti Sains Malaysia
[‡] Universiti Sains Malaysia
[°] Hospital Pulau Pinang

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is an epithelial tumor of the nasopharynx that is rare in most parts of the world, its incidence being less than 1 per 100,000 persons per year worldwide[1] Gravitating towards China, Southeast Asia, Africa, Canada, Alaska and Greenland Eskimos, the highest incidence is observed in the Cantonese-speaking Chinese of Guangdong province in South China, its incidence being 30-50 per 100,000 per year[1] In Malaysia, NPC is the commonest head and neck cancer, and second only to lung cancer among men (constituting 8 8% of total male cancers)[2]. The age standardized incidence in Malaysia for the year of 2003 was 10 2 and 3 6 per 100,000 population for males and females respectively making the male to female ratio 2.75 1 Chinese men had -the highest age standardized incidence rate (18.1 per 100,000 population) followed by Chinese women (7.4 per 100,000 population), Malay males (4.8 per 100,000 population) and Indian males (2 6 per 100,000 population)[2].

NPC, which constitutes 85% of malignant tumors of the nasopharynx, can often be a challenge in diagnosis NPC presents variably, utmost being a neck mass (43-55%), followed by nasal (20-30%), aural (10-17%) and neurologic symptoms (17%)[3,4]. Investigations into these variable manifestations may delay the diagnosis as much as 6 months after the onset of symptoms in 70% of patients[4] A significant proportion (13.3%) have occult primaries at presentation[5] and serial and multiple biopsies are sometimes necessary for a histopathological diagnosis[6]. False negatives may be attributed to submucosal disease, non-representative biopsies and failure to recognize individual malignant cells or small clumps of tumor[7]. Fine-needle aspiration of neck metastases yields a diagnosis only in 82.6% of cases[8] and only in 33.3% of confirmed metastatic carcinoma of the neck with unknown primary could the primary site be found after rigorous diagnostic workup.[9]

The Epstein-Barr virus (EBV) is ubiquitous in NPC, their cells containing multiple copies of the EBV genome regardless of histology or differentiation[10]. The EBV DNA is also found in the preinvasive state of NPC[11] also known as Nasopharyngeal Intraepithelial Neoplasia type III[12] Although EBV is carried by nearly all human adults, B lymphocytes constitute the sole EBV reservoir while persistent infection of epithelial cells is characteristic only of malignant tumors such as NPC[13]. Therefore the detection of genomic DNA in nasopharyngeal biopsy specimens or neck metastases may be highly predictive of NPC[14,15]. Numerous studies performed outside of Southeast Asia have evaluated the role of EBV DNA detection in neck nodes employing PCR and ISH, and have demonstrated a high sensitivity (89-100%) and specificity (78-100%)[14-18]. However, the number of positive NPC patients evaluated have been no more than 18[17] in these studies. The objective of this study is therefore to confirm, using a large sample size, the value of EBV genomic detection in primary site biopsy and neck node aspirates as a diagnostic tool in the region of Malaysia and Southeast Asia

METHODOLOGY

Patients

This evaluation study compared EBV-DNA detection by PCR vs conventional histopathology in primary site biopsy and fine-needle aspirates of neck nodes Specimens were procured with informed consent, following a study protocol approved by regulating bodies.

Eighty-six postnasal biopsies were obtained, with informed consent, from patients who were clinically suspected for NPC Thirty-six were proved histologically to have NPC and fifty were negative for carcinoma. Seventy-one fine-needle aspirates of neck masses were obtained from NPC patients and other neck masses Thirty-six belonged to patients who proved to be NPC by nasopharyngeal biopsy and 35 others consisted of other diagnoses.

NPC histopathology was validated by a pathologist in all patients studied. These patients were recruited from the Otorhinolaryngology clinics of Hospital USM and Hospital Pulau Pinang from July 2002 to September 2003.

The sample size was sufficient to detect a 20% difference (confidence limit = 0 95) between EBV-positive cells from patients and controls with a power of 0 9, a type I error of 0 05, a type II error of 0 1, an odds ratio of 171 and a correlation coefficient of 0.5.

Specimen collection

Tissue was collected during diagnostic biopsies of the nasopharynx and fine-needle aspiration of neck masses and subsequently classified into patient and control groups after histopathological confirmation. In the case of nasopharyngeal biopsies, tissue was obtained from the Fossa of Rossenmuller under endoscopic visualization using a punch biopsy forceps.

The main bulk of tissue was placed in formaldehyde for histopathological examination while a 1-2mm segment of tissue was placed in 1 5mL tubes and stored at -20°C pending tissue diagnosis and laboratory analysis Fine-needle aspirates of neck masses were procured with a 23G needle, 20cc syringe and a biopsy carrier. Similarly, cells were sprayed onto slides for cytological examination and also into a 1 5mL tube for storage pending confirmation and laboratory analysis

Detection of EBV genomic DNA

Tissue specimens were incubated overnight at 56°C in a lysis buffer containing Proteinase K and passed through a silica column. DNA was eluted from the column using a low-salt buffer. We amplified regions of the Epstein-Barr nuclear antigen 1 (EBNA1) (5'- GGT AGA AGG CCA TTT TTC CAC -3' and 5'- CTC CAT CGT CAA AGC TGC AC-3'), nuclear antigen 2 (EBNA2) (5'- CAG GTA CAT GCC AAC AAC CTT -3' and 5'- CCA ACA AAG ATT GTT AGT GGA AT -3') (and the latent membrane protein (LMP) (5'- CAC ATG ACC CGC TGC CTC AT-3' and 5'- CCA ATT CTC GCA TGT CCT CC-3') genes for identification of viral DNA. Amplification of the human beta-actin gene (5'- ATC ATG TTT GAG ACC TTC AAC AC-3' and 5'- CAC CTC TTG CTC GAA GTC CAG -3') provided a marker for the presence of intact genomic DNA. Amplification by PCR method was performed utilizing the above primers and their products analysed on electrophoresed 1% agarose gel containing ethidium bromide as a fluorescent marker. Cloned EBV genomic DNA from a B95-8 cell line was used as a positive control for each test.

Statistical analysis

McNemar's test was used for statistical comparison between matched-pairs and Kappa statistic was used to assess inter-test agreement and reliability Sensitivity, specificity, positive and negative predictive values, likelihood ratio and receiver-operator curves were derived for each gene tested

RESULTS

Of the thirty-six NPC nasopharyngeal biopsy specimens, one was excluded for insufficient DNA. Four were of WHO type 1 classification (11 4%), three WHO type II (8.6%) and 28 WHO type III (80%) Thirty-five histologically negative specimens were selected for controls, 32 of them reported as containing no malignancy and 3 chronic inflammatory changes. That made 35 pairs for comparison in the nasopharyngeal biopsy set. Of the thirty-six NPC neck node aspirates, one was excluded for being a 'vascular tumor' and another excluded for insufficient DNA. From the thirty-five non-NPC neck mass aspirates, one was excluded for insufficient DNA. That made 34 pairs for comparison in the FNAC set.

In the nasopharyngeal biopsy set, 34 out of 35 NPC specimens were positive for EBNA1 gene, while none of the controls were positive 31 out of 35 were positive for the EBNA2 gene, while 2 of the controls were positive. 32 out of 35 were positive for the LMP1 gene, while 4 of the controls were positive. Table 1 shows the validity of detecting each gene in nasopharyngeal biopsy. A p value of >0.05 indicates gene detection being on par with the gold standard of histopathology (i e no significant difference.)

Table 1 - Validity data for EBNA1, EBNA2 and LMP1 gene detection in nasopharyngeal biopsy

	EBNA1	EBNA2	LMP1
Sensitivity	97 14%	88 57%	91 43%

Specificity	100.00%	94.29%	88.57%
Positive predictive value	100.00%	93.94%	88.89%
Negative predictive value	97.22%	89.19%	91.18%
Likelihood ratio	infinity	15 50	8.00
95% Confidence interval	-0.96% to 1.43%	-4.68% to 7.66%	-6 25% to 7 92%
X^2	0 0000	0.1667	0.0000
p value (Mc Nemar's test)	1 000 (>0.05)	0 6831 (>0 05)	1 0000 (>0 05)
Kappa statistic	0 971	0.829	0.800

In the fine-needle aspirate set, 30 out of 34 NPC neck node specimens were positive for EBNA1, while one of the controls were positive 29 out of 34 were positive for the EBNA2 gene, while none of the controls were positive. 30 out of 34 were positive for the LMP1 gene, while 2 of the controls were positive Table 2 shows the validity of detecting each gene in neck node aspirates A p value of >0.05 indicates gene detection being on par with the current standard of a suspicious neck mass in the presence of histopathologically proven primary NPC

Table 2 - Validity data for individual EBV gene detection in fine-needle aspirate of neck masses

	EBNA1	EBNA2	LMP1
Sensitivity	88 24%	85.29%	88.24%
Specificity	97.06%	100 00%	94 12%
Positive predictive value	96 77%	100 00%	93 75%
Negative predictive value	89.19%	87.18%	88.89%
Likelihood ratio	30 00	infinity	15 00
95% Confidence interval	-2 42% to 8 22%	0 70% to 8.70%	-4 21% to 9.23%
X^2	1.5000	4 1667	0.5714
p value	0 2207 (>0 05)	0 0412 (>0.05)	0.4497 (>0.05)
Kappa statistic	0 853	0.853	0.824

Pairwise combination of gene detection in NPC tissue did not offer any advantage in sensitivity and specificity as seen in tables 3 and 4

Table 3 – Comparison of paired gene combinations in postnasal space biopsies

	EBNA1 + EBNA2	EBNA1 + LMP1	EBNA2 + LMP1
Sensitivity	88.57%	91.43%	88.57%
Specificity	100 00%	100.00%	94.29%
Positive predictive value	100.00%	100.00%	93.94%
Negative predictive value	89.74%	92.11%	89.19%
Likelihood ratio	infinity	infinity	15.50
95% Confidence interval	-1.12% to 5 71%	-1.67% to 4.29%	-4.68% to 7.66%
X^2	2.2500	1.3333	0.1667
p value	0 1336 (>0.05)	0 2482 (>0 05)	0.6831 (>0 05)
Kappa statistic	0.886	0.914	0 829

Table 4 – Comparison of paired gene combinations in fine-needle aspirates

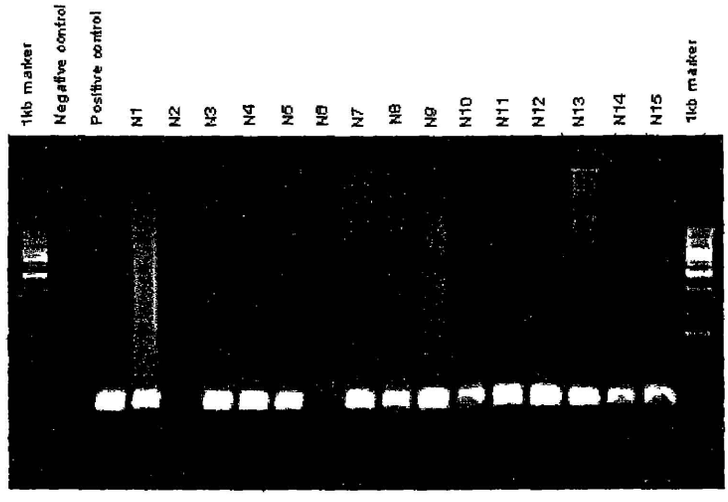
	EBNA1 + EBNA2	EBNA1 + LMP1	EBNA2 + LMP1
Sensitivity	79 41%	82 35%	82.35%
Specificity	100 00%	97.06%	100.00%
Positive predictive value	100.00%	96 55%	100.00%
Negative predictive value	82 93%	84 62%	85.00%
Likelihood ratio	infinity	28 00	infinity
95% Confidence interval	2 99% to 11.59%	-0 61% to 11 11%	1.81% to 10.14%
X^2	6 1250	3 1250	5.1429
p value	0 0133 (<0.05)	0.0771 (>0 05)	0.0233 (<0.05)
Kappa statistic	0 794	0 794	0.824

Analysis of a 'cut-off point' for the total number of genes detected in a nasopharyngeal biopsy specimen, showed a decrease in sensitivity from 97 1% for one gene, to 88 6% for three genes, and an increase in specificity from 88 6% for one gene, to 100% for three genes (p>0.05). Similarly, in fine-needle aspirates, sensitivity for 'cut-off point' decreased from 97.1% for one gene to 79.4% for three genes while specificity increased from 94 1% for one gene to 100% for three genes (p>0.05)

In 11 cases of NPC with clinical neck metastasis (neck node larger than 2cm in levels I and II, or larger than 1cm in levels III-V), PCR was able to detect EBV DNA while FNAC failed to detect any malignancy On the other hand, in one case, PCR failed to detect any of the three genes when FNAC showed metastatic carcinoma. The sensitivity of FNAC in detecting metastatic carcinoma in this sample was 67.6% as opposed to 97 1% by EBV DNA detection. EBV DNA was found in in all histological types of NPC (Table 5).

Table 5 – Histological type and genes detected

	EBNA1	EBNA2	LMP1
WHO type I (4)	4/4	4/4	4/4
WHO type II (3)	3/3	3/3	3/3
WHO type III (28)	27/28	24/28	25/28



Primers: EBNA 1 F&R **Product size: 219bp**

Positive control: B958 EBNA 2 clone 6

Figure 1 Example of an electrophoretic gel for PCR

In one case, an obvious tumour of the nasopharynx could only be positively confirmed by a 2nd biopsy EBV DNA was found in both the first and second biopsy specimens Another patient in the control group, who had no obvious tumor and was

histopatholopgically negative, was dubbed 'falsely positive' for EBV DNA but developed NPC on close follow-up within 1 year.

DISCUSSION

EBNA1 gene detection showed the highest sensitivity and specificity, Kappa value and area under the ROC curve in nasopharyngeal biopsy, and the highest sensitivity in fine-needle aspirates, making it the most suitable and specific gene for the diagnosis of NPC. It is on par with the gold standard of HPE and can provide an adjunct or alternative to diagnosis when it is not forthcoming from histopathological examination. It can also guide the clinician in detecting the primary in cases of unknown primary.

The EBNA1-LMP1 combination is the most suitable pairwise combination of genes for the diagnosis of NPC in nasopharyngeal biopsy but is not superior to the single gene detection of EBNA1. In fine-needle aspirates, the EBNA2-LMP1 combination is the best, but is not on par with the gold standard of histopathology ($p<0.05$) nor is it superior to the single gene detection of EBNA1.

In determining an appropriate 'cut-off point' for nasopharyngeal biopsies, a high sensitivity is desirable for a potentially curable disease such as NPC (while a high specificity is desirable in a disease with grave prognosis) Since there is no statistical difference between the three cut-off points, a >0/3 or >1/3 cut-off points offer the best sensitivity (91.4-97.1%) with an acceptable specificity (88 6-94.3%), but is inferior to single gene detection. In fine-needle aspirates, the >0/3 cut-off point gives the best sensitivity (97.1%) and lowest specificity (94.1%) and is superior to single gene detection (Table 6.)

Table 6- Comparison between single gene, paired gene and cut-off point for postnasal space biopsy and fine-needle aspirate of neck masses

	Sensitivity	Specificity	Inter-test agreement (Kappa)	Area under ROC curve	p value (McNemar's)
N series - Postnasal space biopsy					
Single gene EBNA1	**97.1**	**100**	**0.97**	**0.99**	**>0.05**
Paired gene EBNA1-	91.4	100	,0 91	0 96	>0.05

LMP1					
Cut-off point > 0/3	97.1	88 6	0 77	0 93	>0.05
F series – Fine-needle aspirate of neck mass					
Single gene EBNA1	88.3	97 1	0 85	0 93	>0 05
Paired gene EBNA2-LMP1	82 4	100	0.82	0 91	<0.05
Cut-off point >0/3	**97.1**	**94.1**	**0.91**	**0.96**	**>0.05**

The appearance of EBV DNA in histopathologically negative nasopharyngeal biopsy which subsequently developed NPC may be explained by the presence of EBV DNA in the preinvasive state[11] or NPIN III (Nasopharyngeal intra-epithelial neoplasia stage III)[12]. We conclude that the detection of EBV genes is on par with HPE and that it can be an adjunct to en elusive diagnosis when the diagnosis is missed or when histopathological diagnosis is difficult for whatever reasons – e g non-representative biopsies, submucosal disease, technical difficulties in interpretation [19] It can also predict the development of NPC and prompt serial biopsies on close follow-up

In comparison with other diagnostic modalities, of note is the serum IgA-anti-VCA which has a sensitivity of 97.3% and a specificity of 67 2%. at the >5 serological end-point and IgA-anti-EA-D has a sensitivity of 78.7% and specificity of 97 3% also at the >5 serological end-point 20. Combined at the >5 titre level, together they can reach 80% sensitivity and 98% specificity The limitations of immunofluorescence testing are that it suffers from observer subjectivity, the method must be well controlled and the sensitivity and specificity must be evaluated in each laboratory 21. Exfoliative cytology of the nasopharynx employing a Uterobrush shows a diagnostic rate ranging from 26% to 89% 22. In two studies, PCR on brush biopsy specimens further detects NPC with a sensitivity of at least 87-90% and a specificity of approximately 98-99% 23,24. These studies conclude that the nasopharyngeal swab coupled with PCR-based EBV gene

detection could serve as part of a screening program for high-risk populations. The results of the present study is marginally better though more invasive. EBV gene detection in the postnasal space biopsy can be used when biopsy specimens appear normal on histopathology but are clinically suspicious for NPC It can also predict NPC even when histopathological examination is reported to be normal

The 'occult primary' remains a diagnostic challenge for the clinician. The present study shows a cytological diagnostic accuracy of 67.6% or 76.7% if specimens of insufficient diagnostic material are excluded (11.8%) This is comparable to published figures in Singapore which had a diagnostic accuracy of 82.6% after exclusion of inadequate specimens (which made up 12 2%.)[8] In less than half the cases a primary can be found after rigorous workup which include lymph node biopsy, rigid panendoscopy with systematic biopsies of suspect regions as well as blind biopsies of endoscopically inconspicuous regions, including the tongue base and nasopharynx and bilateral tonsillectomy 8.8% of these were found to originate from the nasopharynx.[25]. In the present study, detection of EBV DNA by PCR in metastatic neck nodes has a far superior diagnostic rate (97.1%) missing only 1 cytologically positive neck node and detected EBV DNA in all cytologically negative aspirates including those with inadequate material PCR is an ideal tool for suggesting NPC and guiding the diagnostic workup in the challenging area of occult primaries, facilitating earlier diagnosis and reducing morbidity and mortality.

CONCLUSION

EBV DNA detection in tissue obtained from postnasal biopsy and fine-needle aspirate of neck masses is a relatively cheap, reliable and accurate method of diagnosing NPC. It is rapid, requires a minimal amount of tissue, and is on par with histopathology in diagnosing NPC and on par with clinico-pathological criteria in detecting metastatic NPC. In nasopharyngeal biopsies it can serve as an adjunct when histopathological diagnosis is missed and also predict the development of NPC It is superior to fine-needle cytology and suggests NPC in the diagnostic workup of occult primaries.

1. Huang DP, Lo KW. Aetiological factors and pathogenesis In Nasopharyngeal Carcinoma Gibb AG, editor 2nd ed· The Chinese University Press; 1999. p. 31.
2. Lim GCC, Yahaya H. SECOND REPORT OF THE NATIONAL CANCER REGISTRY - CANCER INCIDENCE IN MALAYSIA. In: National Cancer Registry, Ministry of Health of Malaysia, 2003. p. 38.
3. van Hasselt CA, Leung SF. Clinical Picture. In· Nasopharyngeal Carcinoma van Hasselt CA, Gibb AG, editors 2nd ed Hong Kong· The Chinese University Press, 1999. p. 105-10.
4. Prasad U In Fifth Malaysian Singapore Congress of Medicine; 1970.
5. Prasad U, Ablashi D, Prathap K, et al. Problem of occult primary in nasopharyngeal carcinoma In· Nasopharyngeal Carcinoma: Current Concepts Prasad U, editor Kuala Lumpur. University of Malaya Press; 1983. p. 11-15

6. Woo J Clinical Diagnosis. In: Nasopharyngeal Carcinoma. van Hasselt CA, Gibb AG, editors 2nd ed. Hong Kong: The Chinese University Press, 1999. p 111-26.
7. Allen PW. Surgical Pathology In: Nasopharyngeal Carcinoma van Hasselt CA, Gibb AG, editors 2nd ed Hong Kong The Chinese University Press; 1999. p. 69-103
8 Kaur A, Chew CT, Lim-Tan SK. Fine needle aspiration of 123 head and neck masses -- an initial experience Ann Acad Med Singapore 1993,22.303-06
9. Haas I, Hoffman TK, Engers R, et al Diagnostic strategies in cervical carcinoma of an unknown primary. Eur Arch Otorhinolaryngol 2002,259.325-33
10. Pathmanathan R, Prasad U, Chandrika G, et al. Undifferentiated, nonkeratinizing, and squamous cell carcinoma of the nasopharynx Variants of Epstein-Barr virus-infected neoplasia. Am J Pathol 1995,146 1355-67.
11 Pathmanathan R, Prasad U, Sadler R, et al Clonal proliferation of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. N Engl J Med 1995;1995 693-98
12 Huang DP, Lo K. Aetiological factors and pathogenesis. In: Nasopharyngeal Carcinoma van Hasselt CA, Gibb AG, editors Hong Kong. The Chinese University Press, 1999 p. 31-60.
13. Niedobitek G, Young LS Epstein-Barr virus persistence and virus-associated tumours Lancet 1994;343:333-5.
14. Feinmesser R, Miyazaki I, Cheung R, et al Diagnosis of nasopharyngeal carcinoma by DNA amplification of tissue obtained by fine-needle aspiration N Engl J Med 1992,326 17-21
15 Feinmesser R, Feinmesser M, Freeman JL, et al. Detection of occult nasopharyngeal primary tumours by means of in situ hybridization J Laryngol Otol 1992;106:345-8.
16 Ohshima K, Kikuchi M, Masuda Y, et al. Epstein-Barr viral genomes in carcinoma metastatic to lymph nodes Association with nasopharyngeal carcinoma Acta Pathol Jpn 1991,41:437-43
17. Walter MA, Menarguez-Palanca J, Peiper SC. Epstein-Barr virus detection in neck metastases by polymerase chain reaction. Laryngoscope 1992;102:481-5.
18. Macdonald MR, Freeman JL, Hui MF, et al. Role of Epstein-Barr virus in fine-needle aspirates of metastatic neck nodes in the diagnosis of nasopharyngeal carcinoma. Head Neck 1995,17:487-93.
19. Allen PWSPIvH, C A , Gibb, A G. (Eds.), Nasopharyngeal Carcinoma 2nd ed. Chinese University Press Surgical Pathology. In: Nasopharyngeal Carcinoma Gibb AG, editor 2nd ed. Chinese University Press, 1999. p. 69-103.
20. Tam JS, Murray HG. Nasopharyngeal carcinoma and Epstein-Barr virus--associated serologic markers. Ear Nose Throat J 1990,69:261-7.
21. Tam JS. Epstein-Barr Virus Serological Markers In: Nasopharyngeal Carcinoma 2nd ed. Gibb AG, editor 2nd ed: Chinese University Press; 1999.
22. Chang AR, Chan MKM. Cytological diagnosis In: Nasopharyngeal Carcinoma 2nd ed. Gibb AG, editor. Chinese University Press, 1999. p. 177-93.
23 Tune CE, Liavaag PG, Freeman JL, et al Nasopharyngeal brush biopsies and detection of nasopharyngeal cancer in a high-risk population J Natl Cancer Inst 1999;91 796-800.

24. Hao SP, Tsang NM, Chang KP. Screening nasopharyngeal carcinoma by detection of the latent membrane protein 1 (LMP-1) gene with nasopharyngeal swabs Cancer 2003;97:1909-13.
25. Haas I, Hoffmann TK, Engers R, et al Diagnostic strategies in cervical carcinoma of an unknown primary (CUP). Eur Arch Otorhinolaryngol 2002;259.325-33.

NOTES

Study protocol approved by the Research Committee of University Sains Malaysia and Medical Research and Ethics Committee of Ministry of Health, Government of Malaysia